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Streptococcus pneumoniae Antigen Test Using Positive Blood Culture Bottles as an Alternative Method To Diagnose Pneumococcal Bacteremia

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Recovery of *Streptococcus pneumoniae* from positive blood culture bottles may be difficult due to autolysis of pneumococci. Therefore, we evaluated the performance of the Binax NOW *S. pneumoniae* antigen test with samples from positive blood culture bottles and defined the duration of detectable pneumococcal antigen in these bottles. Use of the *S. pneumoniae* antigen test is an alternative method for identifying *S. pneumoniae* from positive blood culture bottles and may enable a diagnosis of pneumococcal bacteremia despite negative subcultures.

Pneumococcal bacteremia is an important cause of severe bloodstream infection worldwide and is associated with a high mortality rate (13). Although standard culture-based methods remain the mainstay for diagnosis, the Binax NOW *Streptococcus pneumoniae* antigen test (Binax, Inc., Portland, Maine), an immunochromatographic membrane assay that detects the presence of the C polysaccharide cell wall antigen common to all pneumococcal serotypes, has proven a valuable tool for the rapid diagnosis from urine samples of invasive pneumococcal disease (9, 12). The Food and Drug Administration recently approved the use of this antigen test on cerebrospinal fluid for the rapid diagnosis of pneumococcal meningitis (7).

Although *S. pneumoniae* grows rapidly in most conventional and automated blood culture systems, it produces autolysin, a cell wall enzyme responsible for its own lysis, during its stationary growth phase. Such autolysis may distort the appearance of pneumococci on Gram stain or prevent growth on subculture or both. Identification of *S. pneumoniae* from blood cultures by a nonmolecular method that is not dependent on growth may be useful, particularly when laboratory staffing precludes prompt subculture of positive bottles. Therefore, we evaluated the performance of the Binax NOW *S. pneumoniae* antigen test for detecting pneumococcal antigen and assessed persistence of the antigen in positive blood culture bottles.

All patients with culture-positive blood for *S. pneumoniae* from December 2000 through March 2001 were included in the study. Blood cultures were processed and incubated according to each manufacturer's instructions with the BacT/ALERT (bioMérieux, Durham, NC) or BACTEC 9240 (BD Diagnostic Systems, Sparks, MD) or both automated continuously monitoring instrument systems. Blood culture bottles received in the laboratory during this study period included BacT/ALERT (FA, PF, PF [plastic], FN, and SN) and BACTEC (Plus Aerobic/F and Standard Anaerobic/F) bottles. When the instrument flagged bottles as positive (day 0), an aliquot of blood-broth mixture from positive blood cultures was Gram stained and subcultured onto solid medium according to the results of the Gram stain. Subsequent microbial identification was performed by standard techniques (10). For all cultures identified as *S. pneumoniae*, instrument-positive blood culture bottles were removed from the instrument and held at room temperature. Gram stain and subculture were performed from the instrument-signaled bottles again on days 2 and 7 and at 7-day intervals thereafter until there was no growth on subculture (maximum, 30 days). On days 2, 7, 14, 21, and 30, 2 ml of blood-broth mixture was removed from each instrument-signaled bottle to perform the Binax NOW antigen test. The antigen test was performed according to the manufacturer's instructions for urine samples. A swab was immersed into a 2-ml aliquot of blood-broth mixture from each blood culture bottle and then inserted into the test device. The buffer solution was added, and after 15 min the test was read. The test was interpreted as positive if both sample and control lines were present. The test was interpreted as negative if only the control line was present.

Each companion blood culture bottle that the instrument did not flag positive (negative companion bottle) was removed from the instrument on day 5 and subcultured and had an antigen test performed by day 7. To assess specificity, the antigen test was performed on blood culture bottles collected during the study period that were positive for viridans group streptococci ($n = 12$), beta-hemolytic group A streptococci ($n = 2$), beta-hemolytic group B streptococci ($n = 4$), and nutritionally variant streptococci ($n = 1$).

A total of 56 blood culture bottles from 18 patients with pneumococcal bacteremia were evaluated during the study period. Of 44 (79%) blood culture bottles that the instrument flagged as positive, 42 had a Gram stain smear with gram-

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TABLE 1. Number of positive subculture and antigen results for *S. pneumoniae*

Bottle	No. of positive blood culture bottles						
	Instrument-positive bottles	Subculture day 0	Subculture day 2	Subculture day 7	Subculture day 30	Antigen day 2	Antigen day 30
BACTEC[a]	23	23	20	16	11[b]	23	23
BacT/ALERT[c]	21	20	6	0	0	21	21

[a] Includes BACTEC Plus Aerobic/F and Standard Anaerobic/F bottles.
[b] One additional bottle lost for follow-up.
[c] Includes BacT/ALERT FA, PF glass, PF plastic, FN, and SN bottles.

positive diplococci or cocci in pairs and chains, and 43 grew *S. pneumoniae* on subculture. Subsequent subculture and antigen test results are shown in Table 1. All BacT/ALERT blood culture bottles had negative subcultures on day 7. Of 23 BACTEC bottles that were flagged positive, the pneumococcus was recovered on subculture from 16 bottles on day 7 and from 11 bottles on day 30. All 12 of 56 blood culture bottles that the instrument failed to flag positive had no growth on terminal subculture.

S. pneumoniae antigen was detected in all 43 (100%) blood culture bottles positive for pneumococci at days 2 and 30. *S. pneumoniae* antigen was detected in only one (BacT/ALERT PF plastic) of the seven negative companion bottles tested. Of the 12 blood culture bottles that grew viridans group streptococci, 3 had false-positive pneumococcal antigen tests (Table 2). All three isolates were confirmed to be nonpneumococci by sequencing the full 16S rRNA gene. Pneumococcal antigen was not detected in blood culture bottles that grew beta-hemolytic group A, group B, or nutritionally variant streptococci.

Historically, cultivation of *S. pneumoniae* has been challenging because autolysis resulted in decreased viability. When subcultures were negative, microbiologists often relied on the presence of "chocolatized" medium (the muddy brown sediment that develops in blood culture bottles) or performed laborious latex agglutination tests to diagnose pneumococcal infection (2, 3, 5, 6). The phenomenon of chocolatized medium resulted from a number of complex interactions involving autolysis of pneumococci, hydrogen peroxide production, lowering of the pH, and activation of a hemolysin (5). Advances in automated continuously monitored blood culture systems obviated the need for these methods by enabling laboratorians to process positive bottles before autolysis occurs. Although the problem of false-negative subcultures was not observed in this study, the issue may remain for laboratories with limited staffing and/or resources that preclude prompt subculturing of positive blood culture bottles.

TABLE 2. Antigen results for nonpneumococcal positive blood bottles

Microorganism	No. of bottles positive	No. antigen positive
Viridans group streptococcus	12	3[a]
β-hemolytic group A streptococcus	2	0
β-hemolytic group B streptococcus	4	0
Nutritionally variant streptococcus	1	0

[a] Full 16S rRNA gene sequencing identified isolates as *Streptococcus sanguinis* ($n = 2$) and *Streptococcus mitis* ($n = 1$).

To our knowledge no publications have reported studies of the Binax NOW *S. pneumoniae* antigen test for detecting the presence of pneumococcal antigen in blood culture bottles. We detected pneumococcal antigen in 100% of blood culture bottles that grew *S. pneumoniae*. Also, pneumococcal antigen was detected for 30 days following initial subculture for all bottles. False-positive reactions may occur, and several authors have demonstrated that viridans group streptococci, in particular the *Streptococcus mitis* group, may cross-react with the pneumococcal C polysaccharide antigen (1, 4, 8, 11). All three false-positive results in our study were due to organisms within the *S. mitis* group.

The Binax NOW antigen test is useful for detecting pneumococcal antigen in positive blood culture bottles that have a Gram stain smear compatible with *S. pneumoniae*. In the appropriate clinical setting, particularly when subculture is negative or delayed due to limited staffing, the pneumococcal antigen test may be used as an alternative method for making a presumptive diagnosis of pneumococcal bacteremia.

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